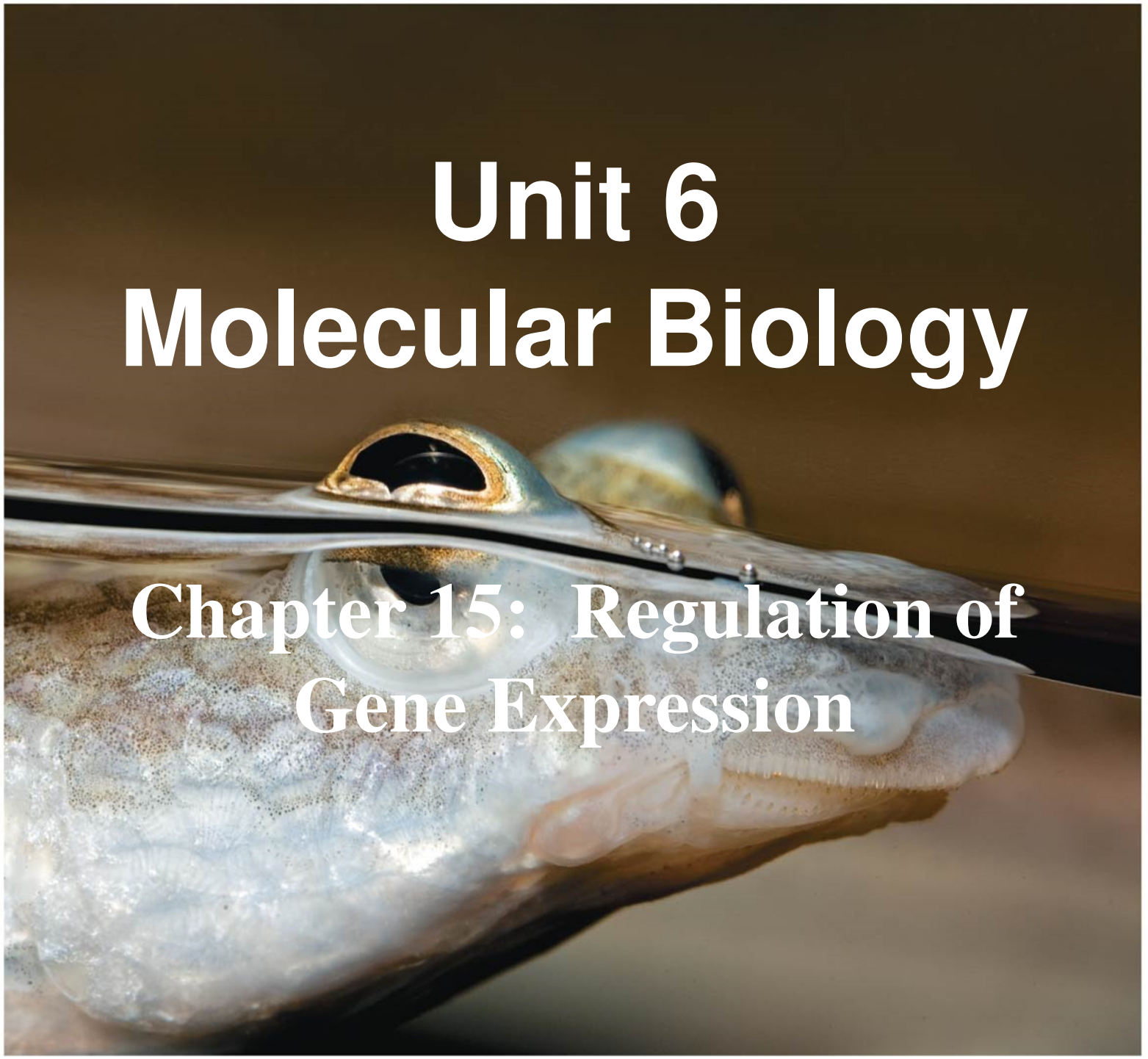


Unit 6
Molecular Biology

Chapter 15: Regulation of Gene Expression

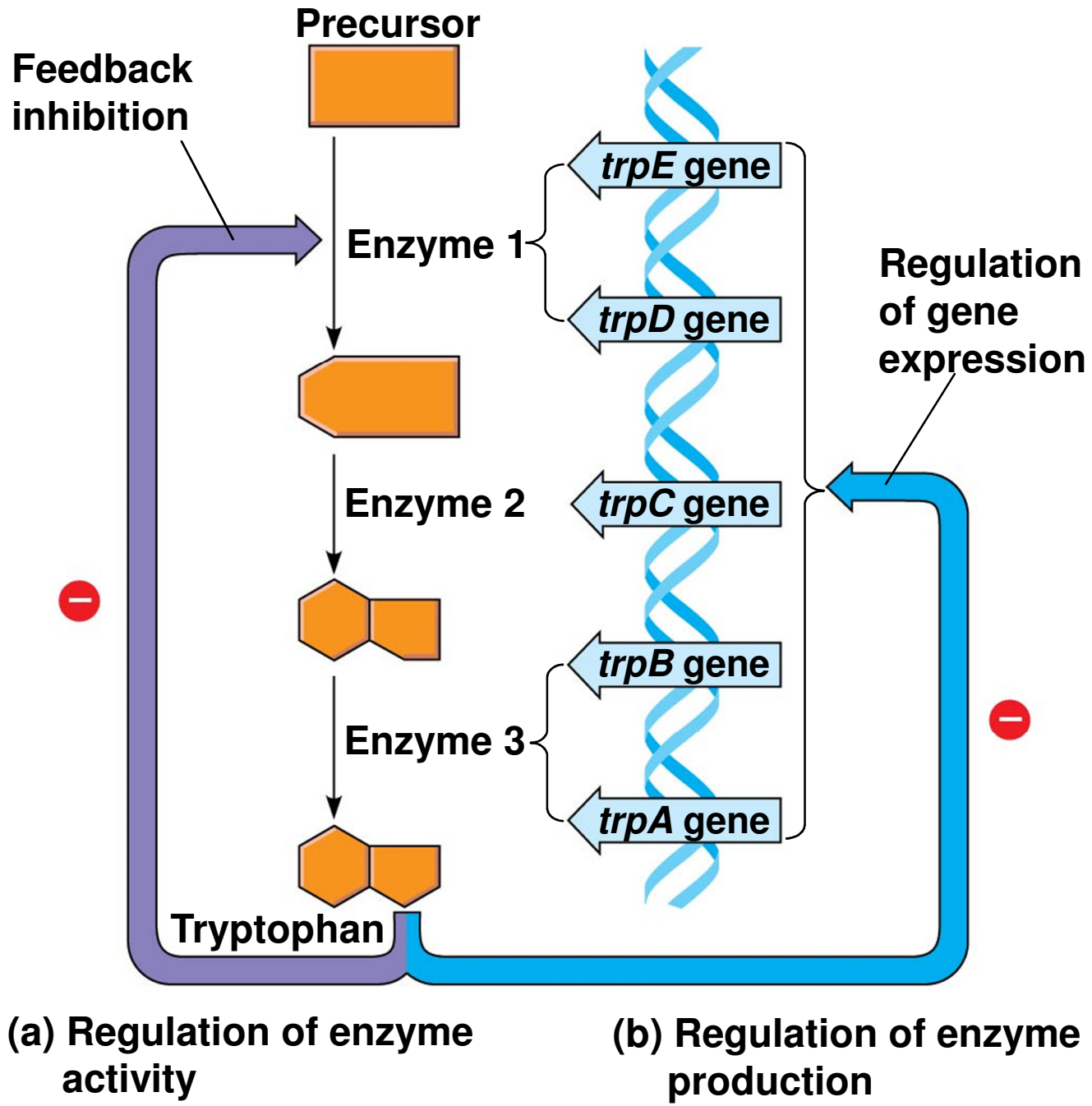
Overview: Differential Expression of Genes

- Prokaryotes and eukaryotes alter gene expression in response to their changing environment
- Multicellular eukaryotes also develop and maintain multiple cell types
- Gene expression is often regulated at the transcription stage, but control at other stages is important, too

Concept 15.1: Bacteria often respond to environmental change by regulating transcription

- Natural selection has favored bacteria that produce only the gene products needed by the cell
- A cell can regulate production of enzymes on 2 levels
  1. *Feedback inhibition* (rapid response)
     - Enzyme inhibited by accumulation of its end product
     - Allows a cell to adapt to short-term fluctuations in the supply of a needed substance
  2. Gene regulation (longer-term response)
     - Transcription of the mRNA coding for the enzyme is switched on or off
- Gene expression in bacteria is controlled by a mechanism described as the *operon model*

Figure 15.2


Precursor
Feedback inhibition
Enzyme 1
Enzyme 2
Enzyme 3
Tryptophan
trpE gene
trpD gene
trpC gene
trpB gene
trpA gene
Regulation of gene expression
(a) Regulation of enzyme activity
(b) Regulation of enzyme production

Operons: The Basic Concept

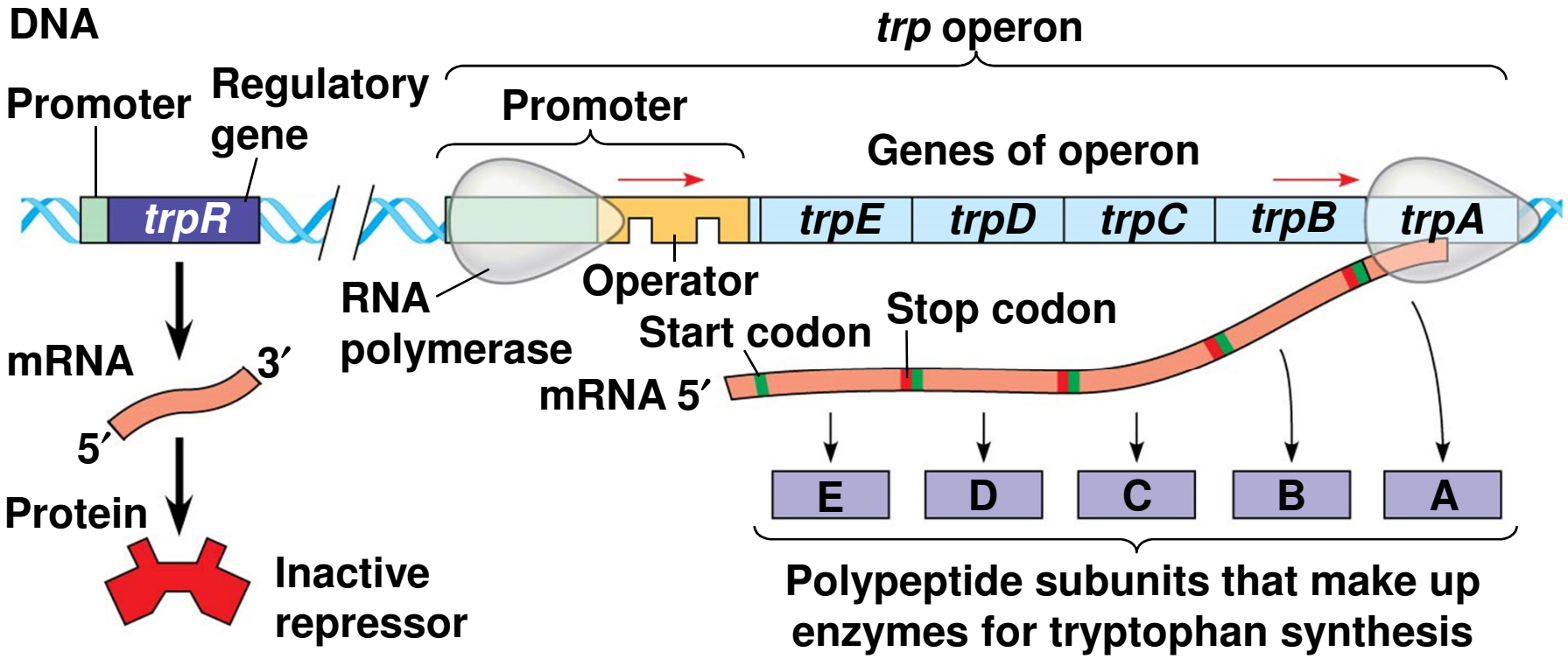
- A group of functionally related genes can be coordinately controlled by a single "on-off switch"
- The regulatory "switch" is a segment of DNA called an **operator** usually positioned within the promoter
  - Controls access of RNA polymerase to the genes
- An **operon** is the entire stretch of DNA that includes the operator, the promoter, and the genes that they control

- The operon can be switched off by a protein **repressor**
- The repressor prevents gene transcription by binding to the operator and blocking RNA polymerase
  - Repressor is specific for the operator of a particular operon
- The repressor is the product of a separate **regulatory gene**

- The binding of repressors to operators is reversible
- The repressor is often an allosteric protein with two alternative shapes
  - An active or inactive form, depending on the presence of other molecules
- A **corepressor** is a molecule that cooperates with a repressor protein to switch an operon off

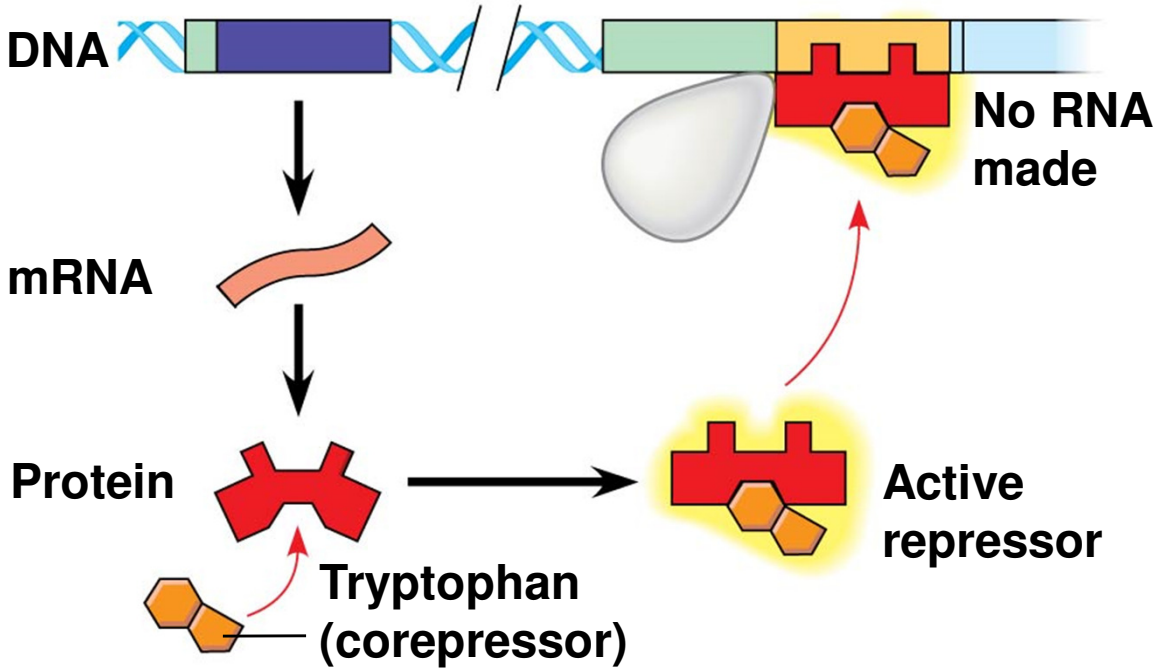
- By default the *trp* operon in *E. coli* is on and the genes for tryptophan synthesis are transcribed
- When tryptophan is present, it binds to the *trp* repressor protein, which then turns the operon off
- The repressor is active only in the presence of its corepressor tryptophan
  - Thus the *trp* operon is turned off (repressed) if tryptophan levels are high

Figure 15.3a



(a) Tryptophan absent, repressor inactive, operon on

Figure 15.3b



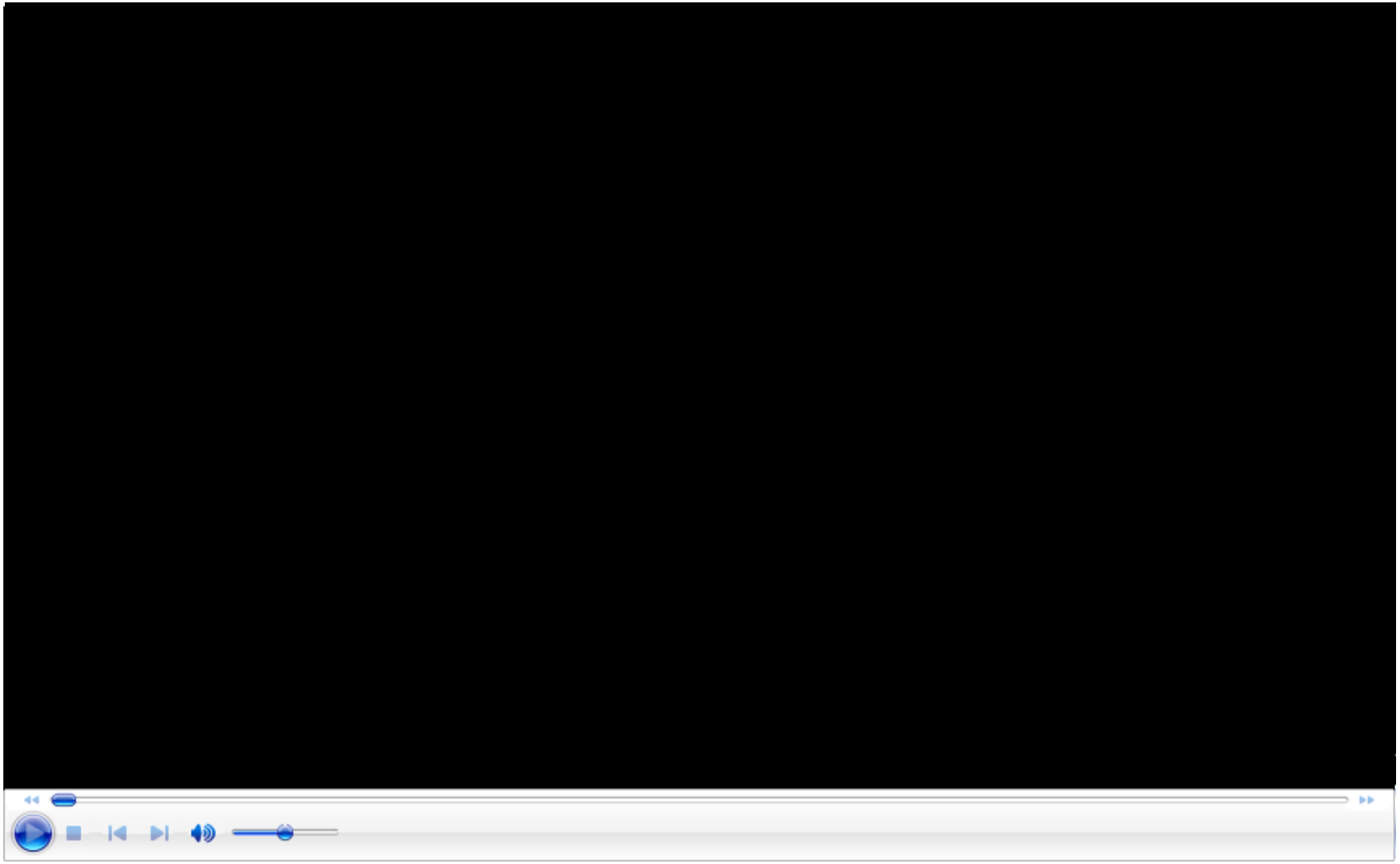
(b) Tryptophan present, repressor active, operon off

Repressible and Inducible Operons: Two Types of Negative Gene Regulation

- A *repressible operon* is one that is usually on
  - Binding of a repressor to the operator shuts off transcription
  - Generally function in anabolic pathways
    - Synthesize essential end products from raw materials
  - Their synthesis is repressed by high levels of the end product
  - The *trp* operon is a repressible operon

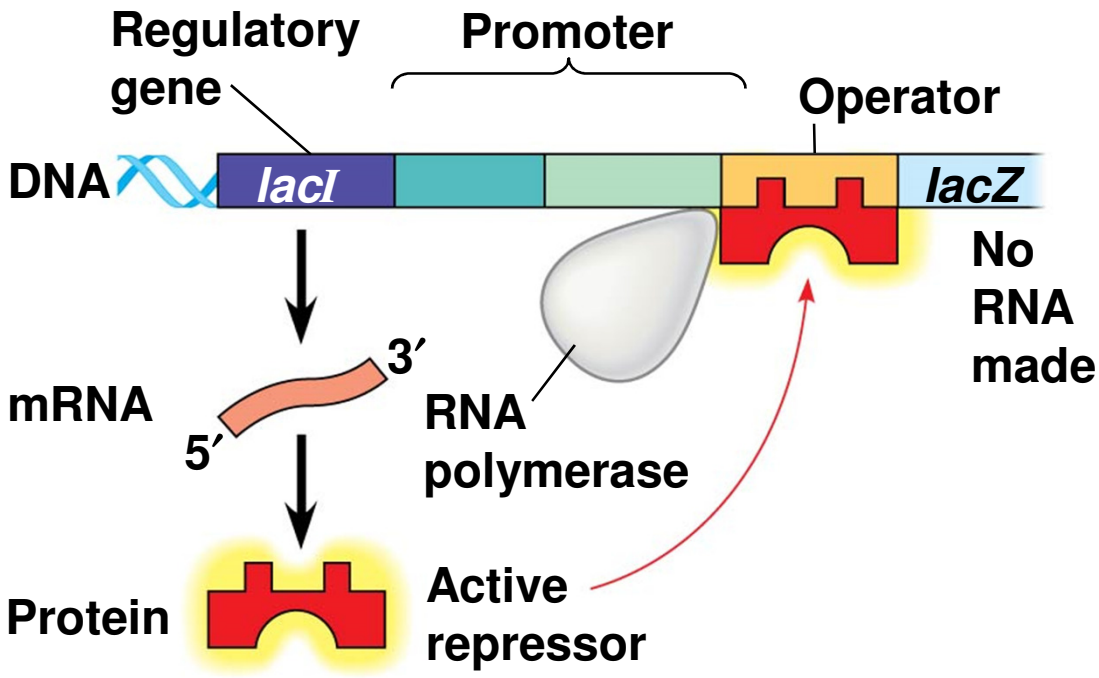
- An *inducible operon* is one that is usually off
  - A molecule called an inducer inactivates the repressor and turns on transcription
  - Generally function in catabolic pathways
    - Break down nutrients into simpler molecules
  - Synthesis is induced by a chemical signal
  - The *lac* operon is an inducible operon

- The *lac* operon contains genes that code for enzymes used in the hydrolysis and metabolism of lactose
- By itself, the *lac* repressor is active and switches the *lac* operon off
- A molecule called an **inducer** inactivates the repressor to turn the *lac* operon on
  - For the *lac* operon, the inducer is allolactose, formed from lactose that enters the cell



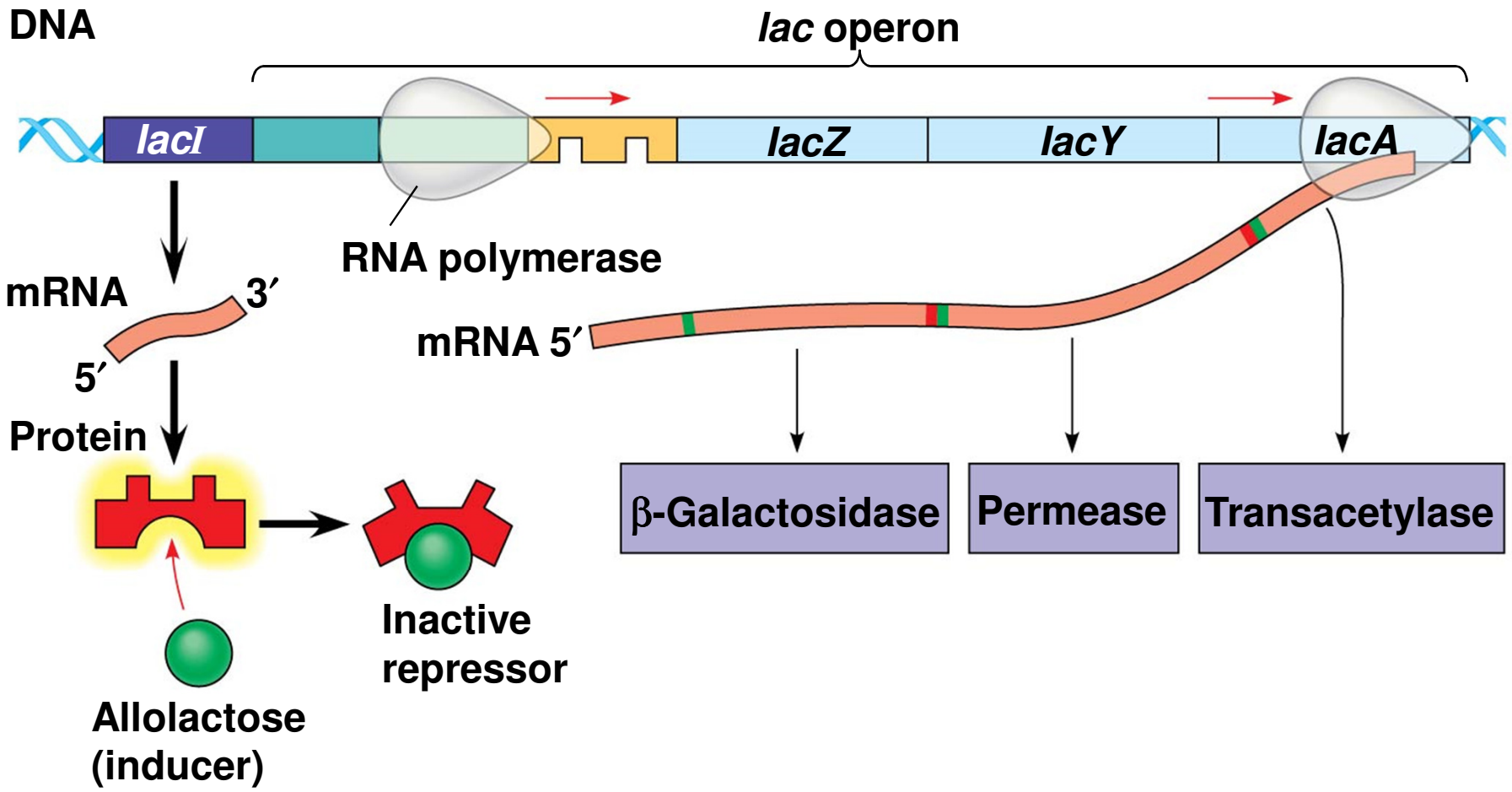
Video: *lac* Repressor Model

Figure 15.4a



(a) Lactose absent, repressor active, operon off

Figure 15.4b



(b) Lactose present, repressor inactive, operon on

- Enzymes of the lactose pathway are called inducible enzymes
- Analogously, the enzymes for tryptophan synthesis are said to be repressible enzymes

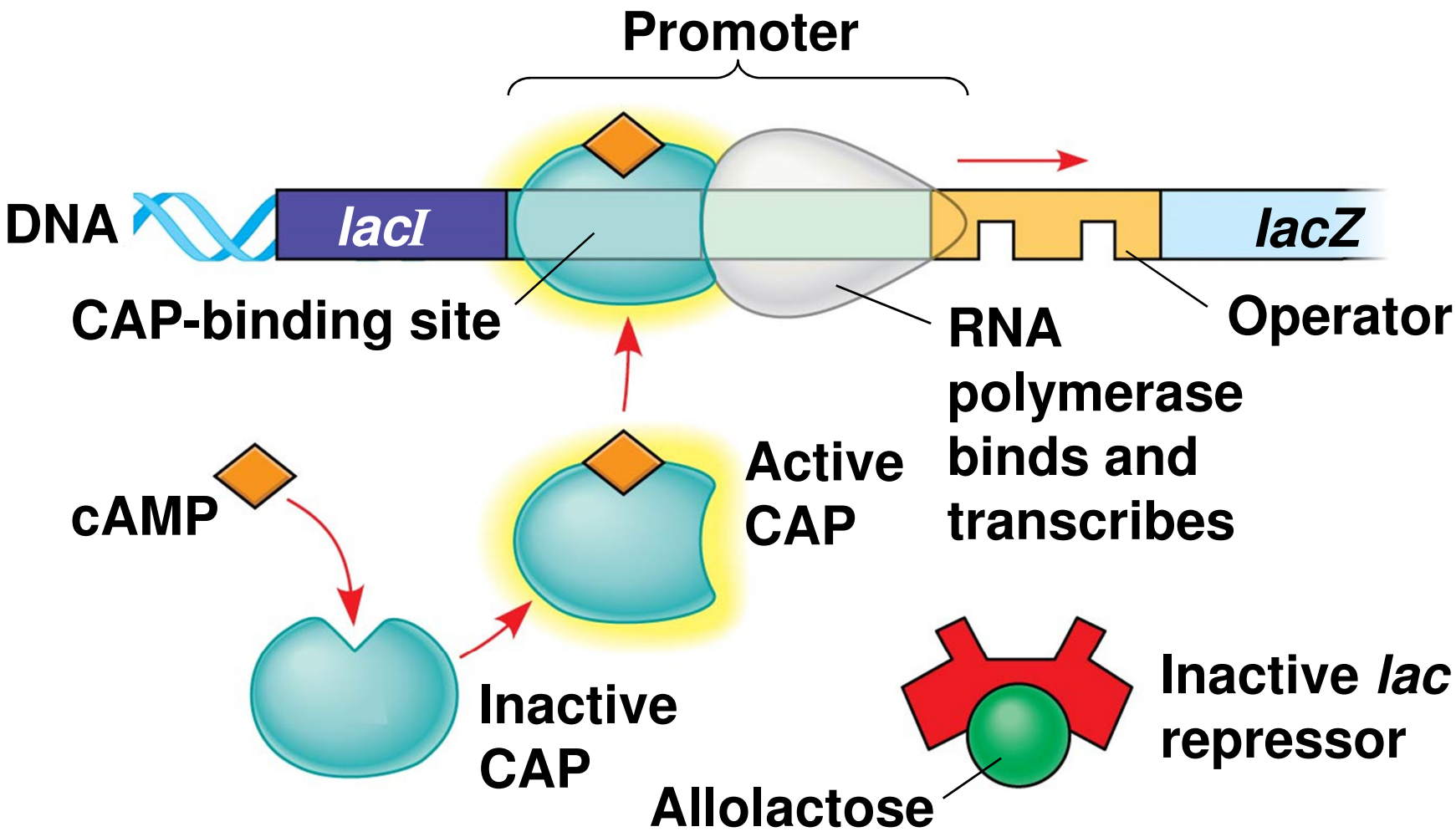
- Regulation of the *trp* and *lac* operons involves *negative* control of genes because operons are switched off by the active form of the repressor
- Gene regulation is said to be *positive* when a regulatory protein interacts directly with the genome to switch transcription on

Positive Gene Regulation

- *E. coli* will preferentially use glucose when it is present in the environment
- When glucose is scarce, CAP (catabolite activator protein) acts as an **activator**
  - Protein that binds to DNA and stimulates transcription of a gene
- CAP is activated by binding with **cyclic AMP (cAMP)**
- Activated CAP attaches to the promoter of the *lac* operon and increases the affinity of RNA polymerase
  - Thus accelerates transcription

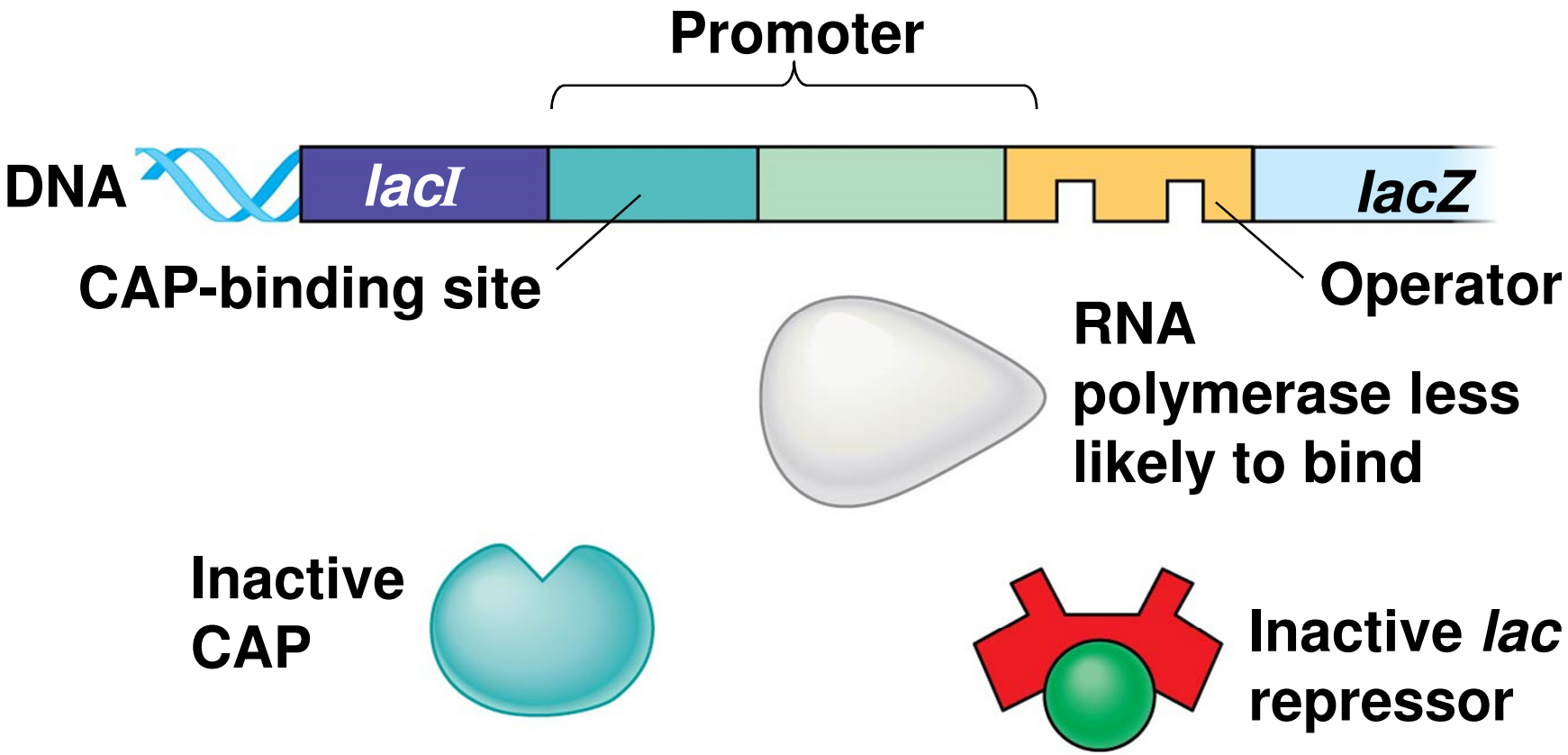
- When glucose levels increase
  - CAP detaches from the *lac* operon
  - Transcription proceeds at a very low rate, even if lactose is present
- Thus, *lac* operon is under dual control
  - Negative control by the *lac* repressor
    - Determines whether or not transcription occurs
  - Positive control by CAP
    - Controls rate of transcription
- CAP helps regulate other operons that encode enzymes used in catabolic pathways

Figure 15.5a



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized

Figure 15.5b



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

Concept 15.2: Eukaryotic gene expression is regulated at many stages

- All organisms must regulate which genes are expressed at any given time
- In multicellular organisms regulation of gene expression is essential for cell specialization

Differential Gene Expression

- Almost all the cells in an organism are genetically identical
- Differences between cell types result from **differential gene expression**
  - The expression of different genes by cells with the same genome
- Abnormalities in gene expression can lead to diseases, including cancer
- Gene expression is regulated at many stages
  - In all organisms, a common control point for gene expression is at transcription

Figure 15.6a

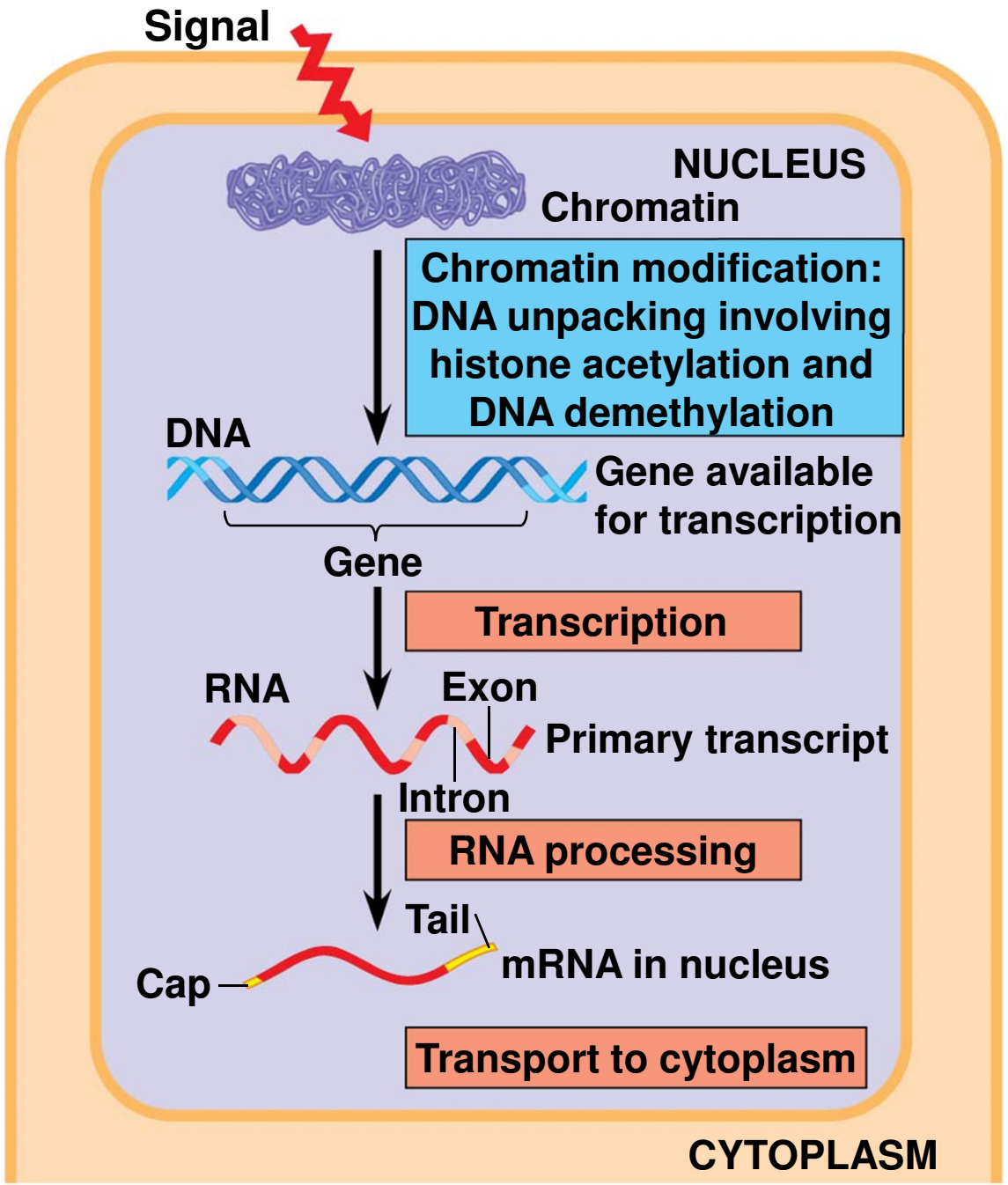
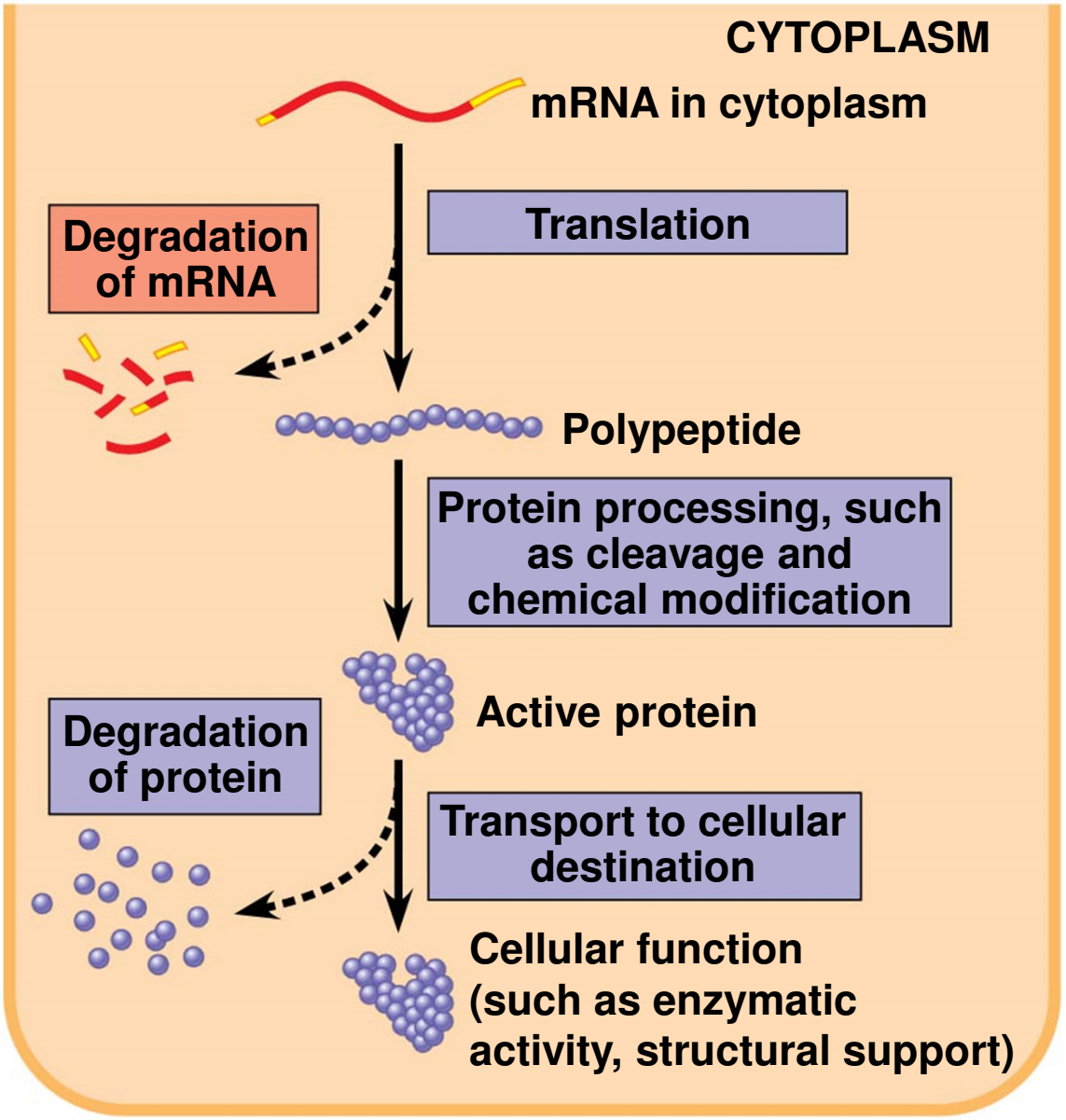

Signal
NUCLEUS
Chromatin
Chromatin modification: DNA unpacking involving histone acetylation and DNA demethylation
DNA
Gene available for transcription
Gene
Transcription
RNA
Exon
Primary transcript
Intron
RNA processing
Tail
Cap
mRNA in nucleus
Transport to cytoplasm
CYTOPLASM

Figure 15.6b


CYTOPLASM
mRNA in cytoplasm
Translation
Degradation of mRNA
Polypeptide
Protein processing, such as cleavage and chemical modification
Active protein
Degradation of protein
Transport to cellular destination
Cellular function (such as enzymatic activity, structural support)

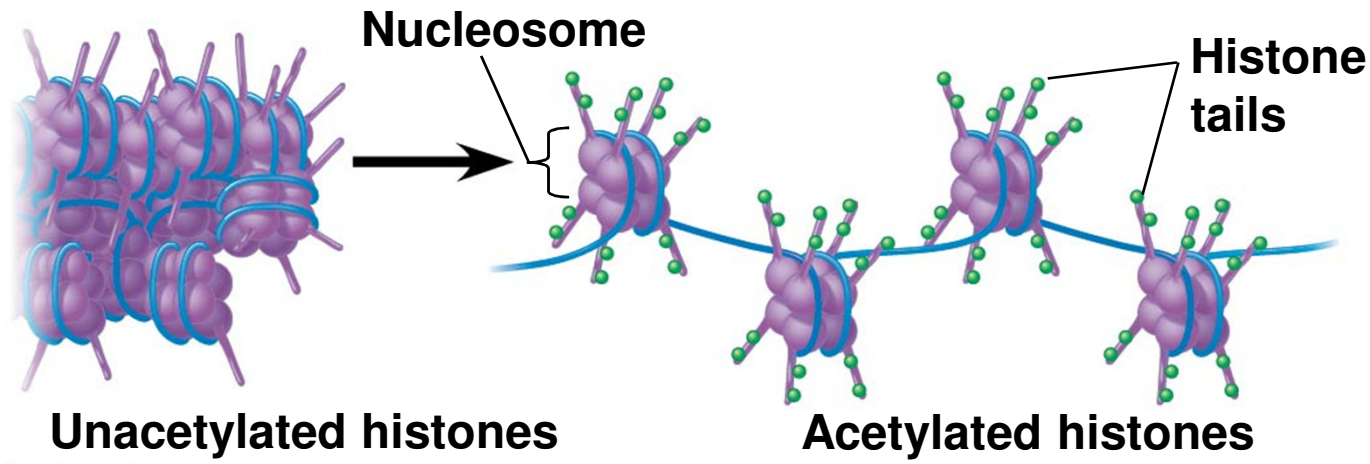
Regulation of Chromatin Structure

- The structural organization of chromatin packs DNA into a compact form and also helps regulate gene expression in several ways
- The location of a gene promoter relative to nucleosomes and scaffold or lamina attachment sites can influence gene transcription
- Genes within highly condensed heterochromatin are usually not expressed
- Chemical modifications to histone proteins and DNA can influence chromatin structure and gene expression

Histone Modifications and DNA Methylation

- Remember, histones are the proteins around which DNA is wrapped in nucleosomes
  - Chemical modifications to histones play a direct role in the regulation of gene transcription
- In **histone acetylation**, acetyl groups are attached to positively charged lysines in histone tails
  - This generally loosens chromatin structure
  - Thus promoting the initiation of transcription
- The addition of methyl groups (methylation) can condense chromatin
  - Leads to reduced transcription

Figure 15.7


Nucleosome
Histone tails
Unacetylated histones
Acetylated histones

- **DNA methylation** is the addition of methyl groups to certain bases in DNA, usually cytosine
  - Individual genes are usually more heavily methylated in cells where they are not expressed
  - Once methylated, genes usually remain so through successive cell divisions
  - After replication, enzymes methylate the correct daughter strand so that the methylation pattern is inherited

Epigenetic Inheritance

- Though chromatin modifications do not alter DNA sequence, they may be passed to future generations of cells
- The inheritance of traits transmitted by mechanisms not directly involving the nucleotide sequence is called **epigenetic inheritance**
- Epigenetic modifications can be reversed, unlike mutations in DNA sequence

Regulation of Transcription Initiation

- Chromatin-modifying enzymes provide initial control of gene expression by making a region of DNA either more or less able to bind the transcription machinery
- The regulation of transcription initiation involves proteins that bind to DNA and either facilitate or inhibit binding of RNA polymerase
  - More complicated in eukaryotes than prokaryotes

Organization of a Typical Eukaryotic Gene

- Associated with most eukaryotic genes are multiple **control elements**
  - Segments of noncoding DNA that serve as binding sites for transcription factors that help regulate transcription
- Control elements and the transcription factors they bind are critical for the precise regulation of gene expression in different cell types

Figure 15.8a

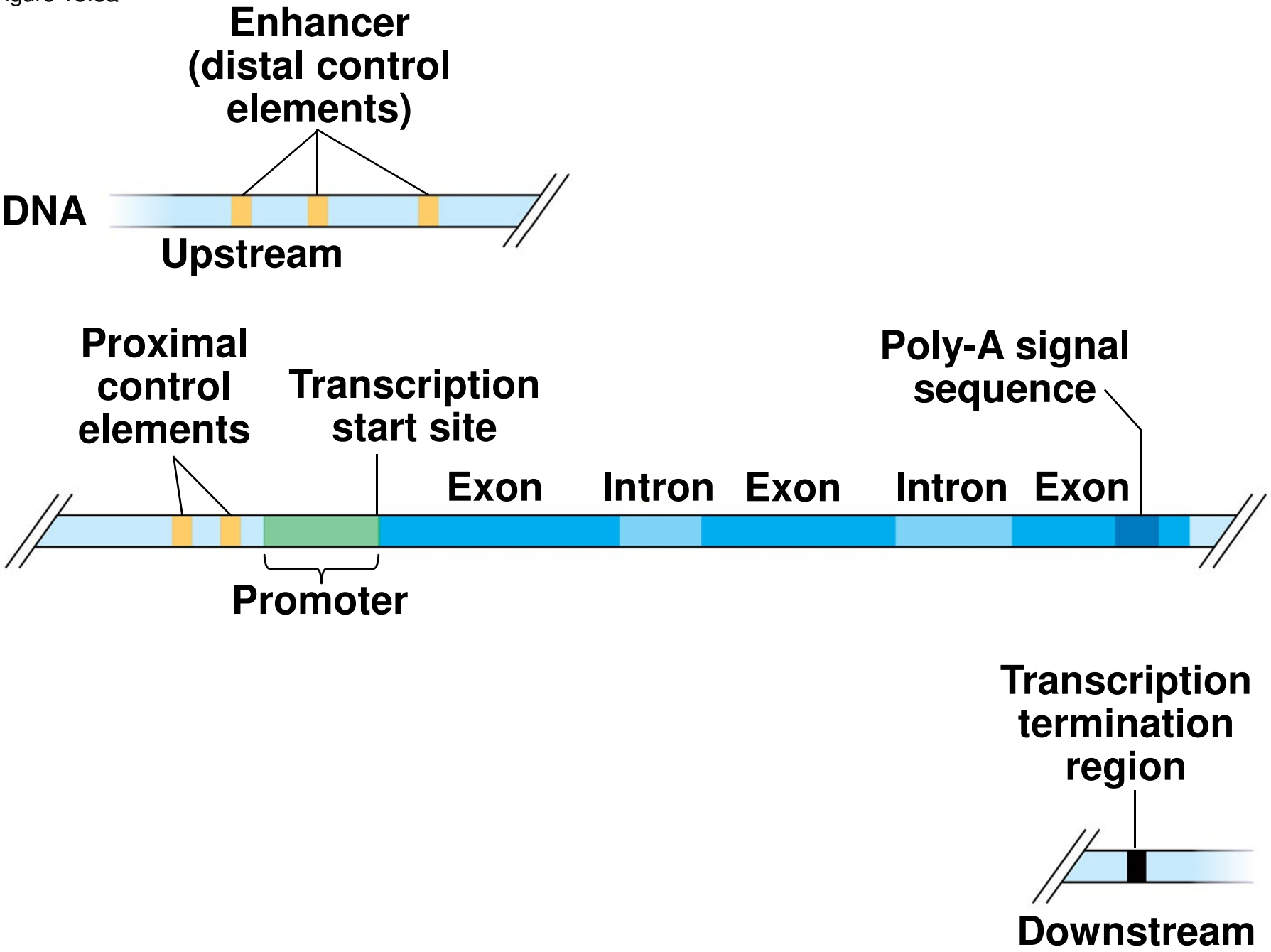
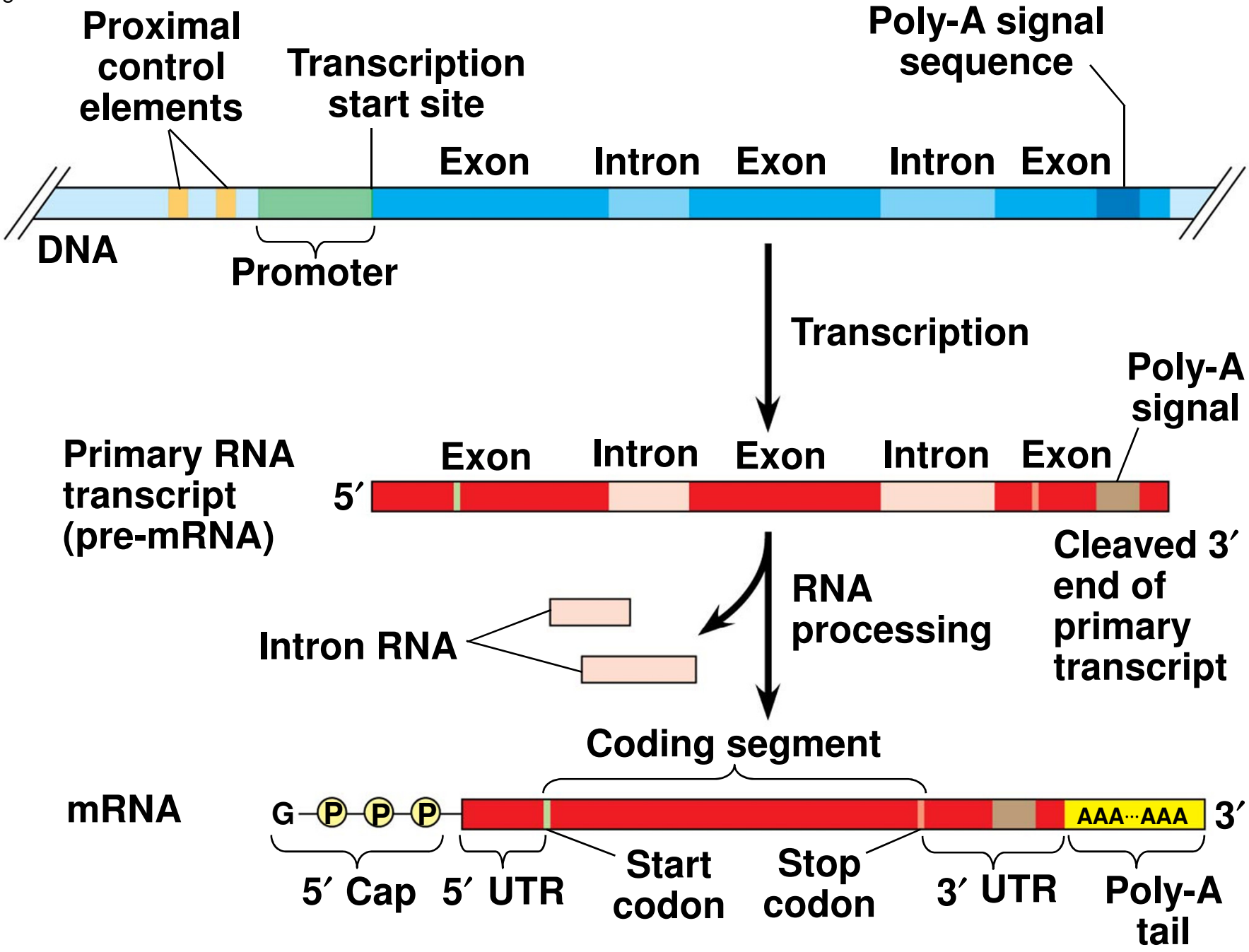

Enhancer
(distal control
elements)
DNA
Upstream
Proximal
control
elements
Transcription
start site
Poly-A signal
sequence
Exon
Intron
Exon
Intron
Exon
Promoter
Transcription
termination
region
Downstream

Figure 15.8b-3



The Roles of Transcription Factors

- To initiate transcription, eukaryotic RNA polymerase requires the assistance of proteins called transcription factors
  - *General transcription factors* are essential for the transcription of all protein-coding genes
- In eukaryotes, high levels of transcription of particular genes depend on interaction between control elements and *specific transcription factors*

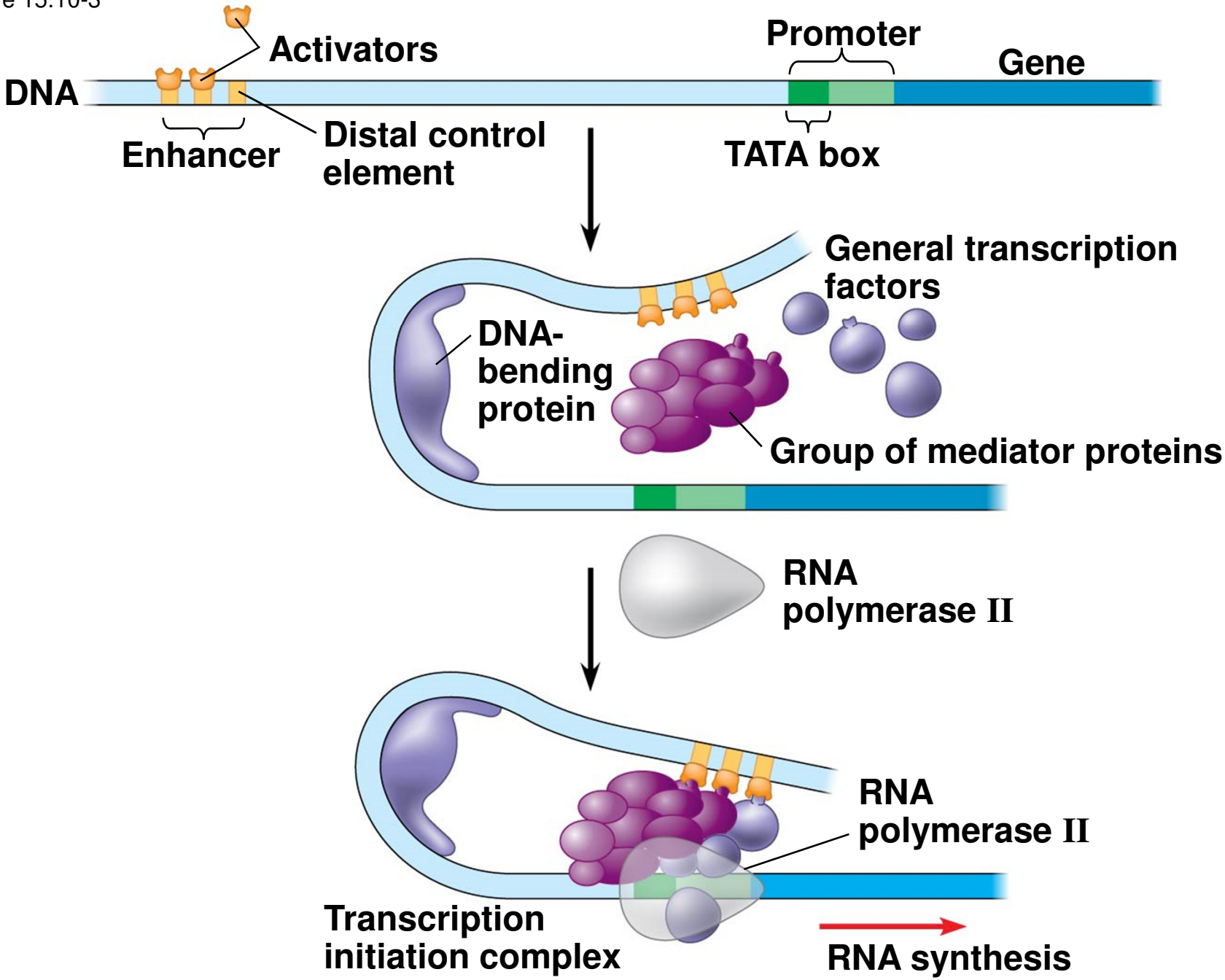
Enhancers and Specific Transcription Factors

- *Proximal control elements* are located close to the promoter
- *Distal control elements*, groupings of which are called **enhancers**, may be far away from a gene or even located in an intron

- The rate of gene expression can be strongly increased or decreased
  - By binding of specific transcription factors to control elements of enhancers
    - Can be either activators or repressors

- An activator is a protein that binds to an enhancer and stimulates transcription of a gene
  - Activators have two domains
    - One that binds DNA
    - A second that activates transcription
- Some transcription factors function as repressors
  - Inhibit expression of a particular gene by a variety of methods
- Some activators and repressors act indirectly by influencing chromatin structure to promote or *silence* transcription

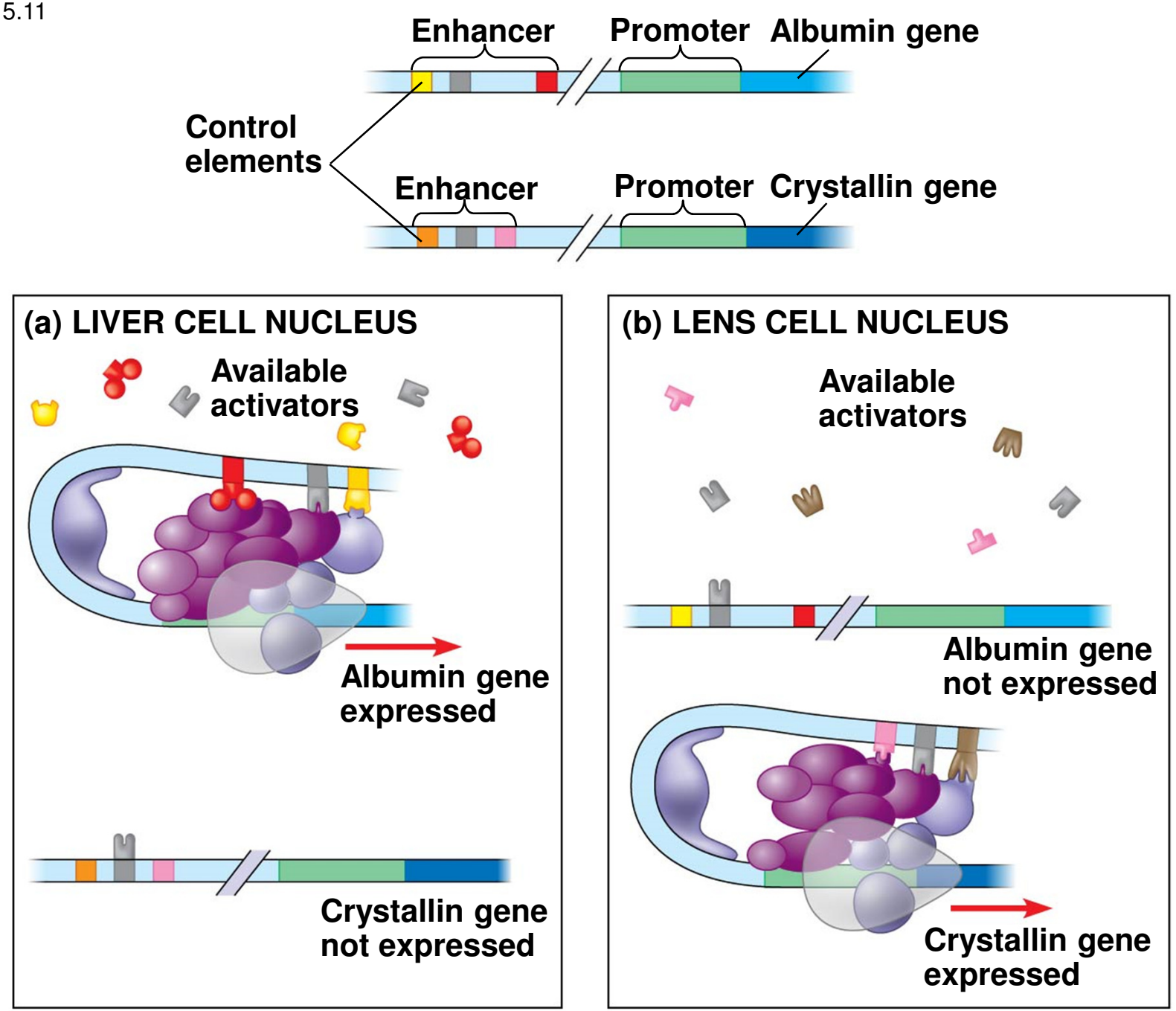
Figure 15.10-3



Combinatorial Control of Gene Activation

- A particular *combination* of control elements can activate transcription only when the appropriate activator proteins are present

Figure 15.11


Enhancer
Promoter
Albumin gene
Control elements
Enhancer
Promoter
Crystallin gene
(a) LIVER CELL NUCLEUS
Available activators
Albumin gene expressed
Crystallin gene not expressed
(b) LENS CELL NUCLEUS
Available activators
Albumin gene not expressed
Crystallin gene expressed

Coordinately Controlled Genes in Eukaryotes

- Unlike the genes of a prokaryotic operon, EACH of the co-expressed eukaryotic genes has a promoter and control elements
  - These genes can be scattered over different chromosomes
  - But each has the same combination of control elements
- Copies of the activators recognize specific control elements and promote simultaneous transcription of the genes

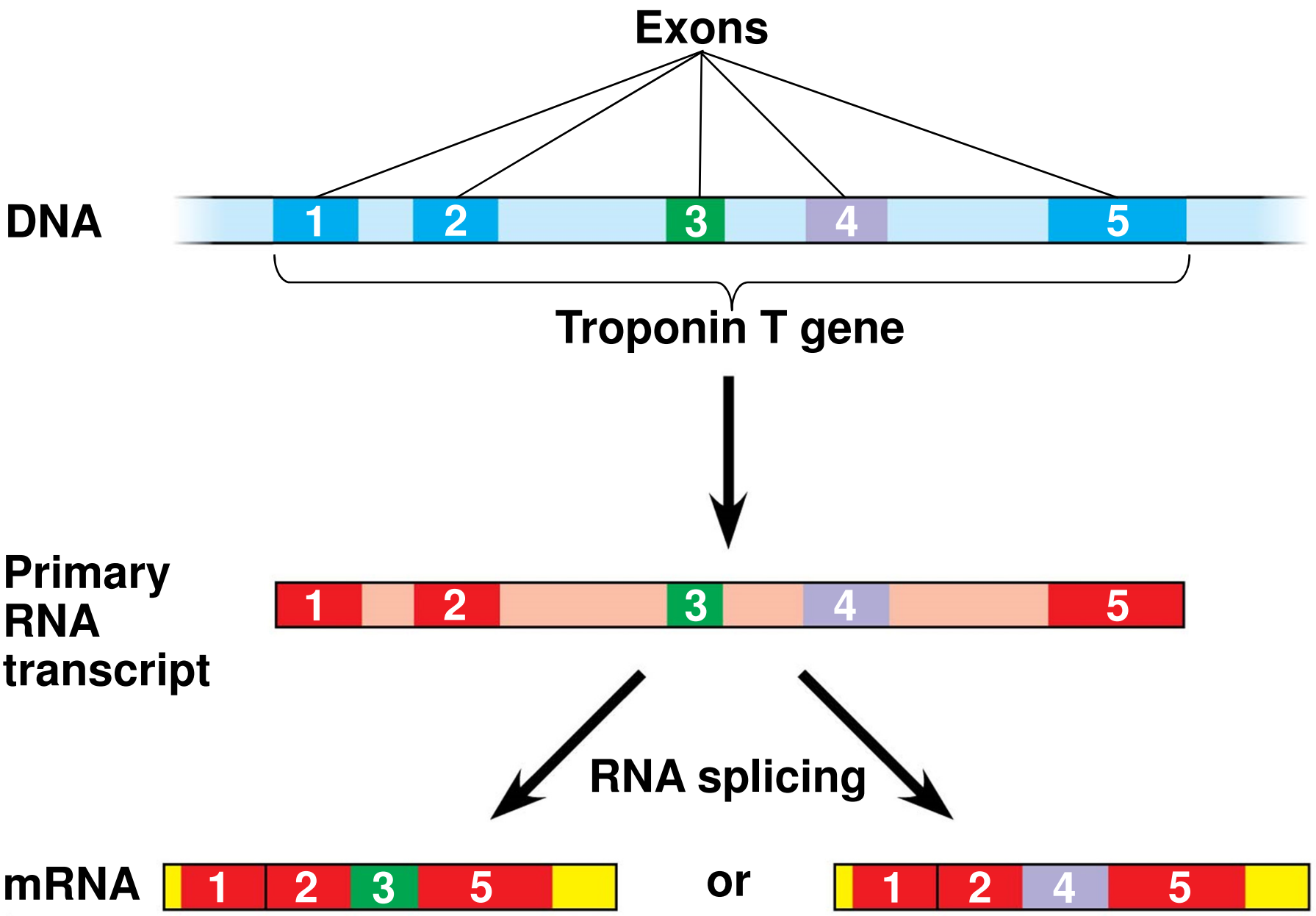
Mechanisms of Post-Transcriptional Regulation

- Transcription alone does not account for gene expression
- Regulatory mechanisms can operate at various stages after transcription
- Such mechanisms allow a cell to fine-tune gene expression rapidly in response to environmental changes

RNA Processing

- In **alternative RNA splicing**, different mRNA molecules are produced from the same primary transcript
  - Depending on which RNA segments are treated as exons and which as introns
- Alternative splicing was proposed as one explanation for the surprisingly low number of human genes

Figure 15.12


Exons
DNA
1
2
3
4
5
Troponin T gene
Primary RNA transcript
1
2
3
4
5
RNA splicing
mRNA
1
2
3
5
or
1
2
4
5

mRNA Degradation

- The life span of mRNA molecules in the cytoplasm is important in determining the pattern of protein synthesis in a cell
- Eukaryotic mRNA generally survives longer than prokaryotic mRNA
- Nucleotide sequences that influence the life span of mRNA in eukaryotes reside in the untranslated region (UTR) at the 3′ end of the molecule

Initiation of Translation

- Translation regulation occurs most commonly at initiation stage
  - The initiation of translation of selected mRNAs can be blocked by regulatory proteins that bind to sequences or structures of the mRNA
  - Prevents attachment of ribosomes
- Alternatively, translation of ALL mRNAs in a cell may be regulated simultaneously
  - For example, translation initiation factors are simultaneously activated in an egg following fertilization

Protein Processing and Degradation

- After translation, various types of protein processing, including cleavage and chemical modification, are subject to control
- The length of time each protein functions in a cell is regulated by means of selective degradation
- To mark a particular protein for destruction, the cell commonly attaches molecules of ubiquitin to the protein, which triggers its destruction

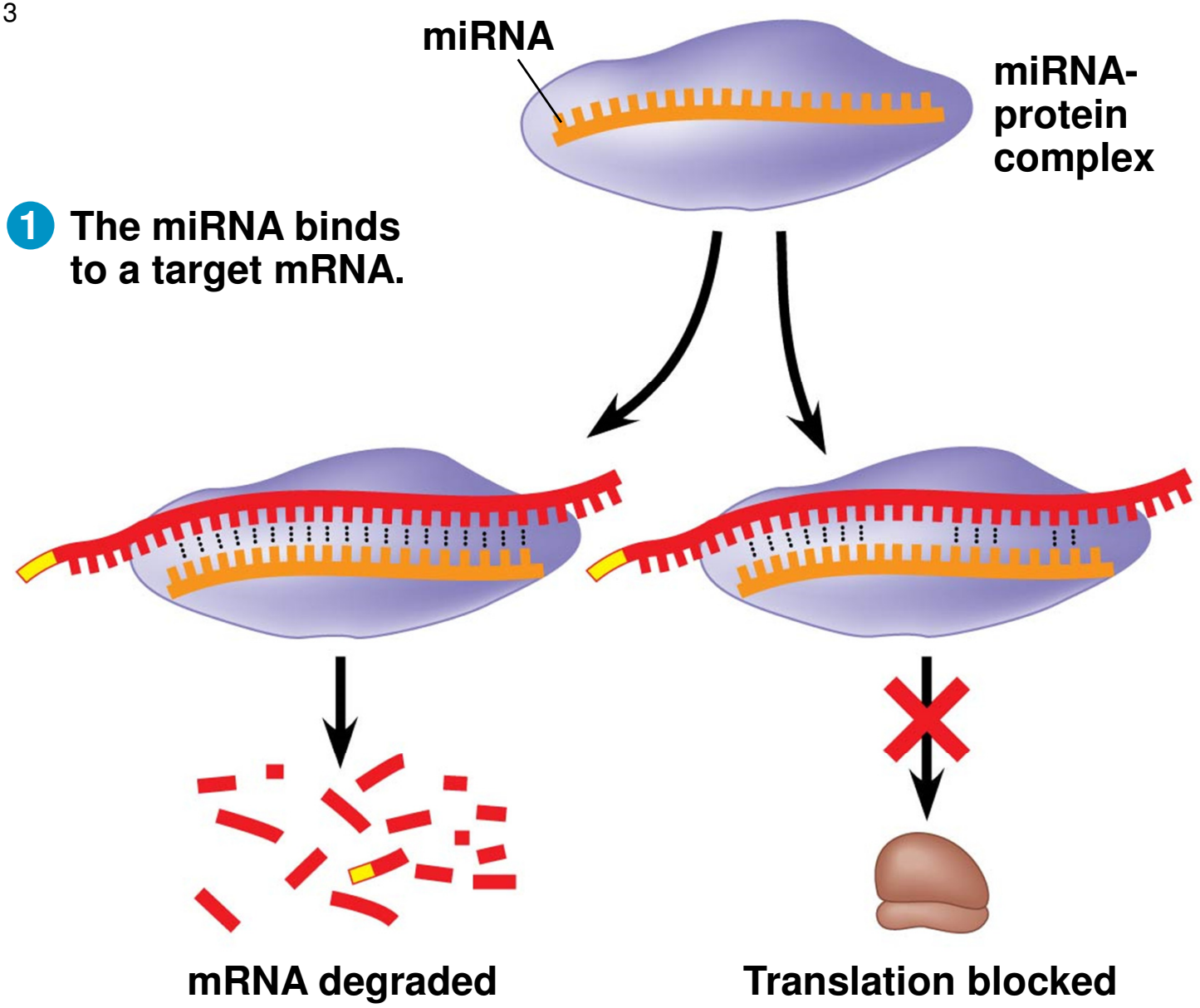
Concept 15.3: Noncoding RNAs play multiple roles in controlling gene expression

- Only a small fraction of DNA encodes proteins
- A very small fraction of the non-protein-coding DNA consists of genes for RNA such as rRNA and tRNA
- A significant amount of the genome may be transcribed into *noncoding RNAs (ncRNAs)*
  - Noncoding RNAs regulate gene expression at several points

Effects on mRNAs by MicroRNAs and Small Interfering RNAs

- **MicroRNAs** (**miRNAs**) are small single-stranded RNA molecules that can bind to complementary mRNA sequences
  - These can degrade the mRNA or block its translation
  - At least ½ of all human genes may be regulated by miRNAs
- Another class of small RNAs are called **small interfering RNAs** (**siRNAs**)
  - The blocking of gene expression by siRNAs is called **RNA interference** (**RNAi**)

Figure 15.13


miRNA
miRNA-protein complex
1 The miRNA binds to a target mRNA.
mRNA degraded
Translation blocked
2 If bases are completely complementary, mRNA is degraded. If match is less than complete, translation is blocked.

Chromatin Remodeling and Effects on Transcription by ncRNAs

- In some yeasts RNA produced from centromeric DNA is copied into double-stranded RNA and then processed into siRNAs
- The siRNAs, together with a complex of proteins, act as a homing device to target transcripts being made from centromeric sequences
- Proteins in the complex then recruit enzymes that modify the chromatin to form the highly condensed heterochromatin found at the centromere

- A class of small ncRNAs called piwi-associated RNAs (piRNAs) also induce formation of heterochromatin
- They block expression of transposons, parasitic DNA elements in the genome
- The role of ncRNAs adds to the complexity of the processes involved in regulation of gene expression

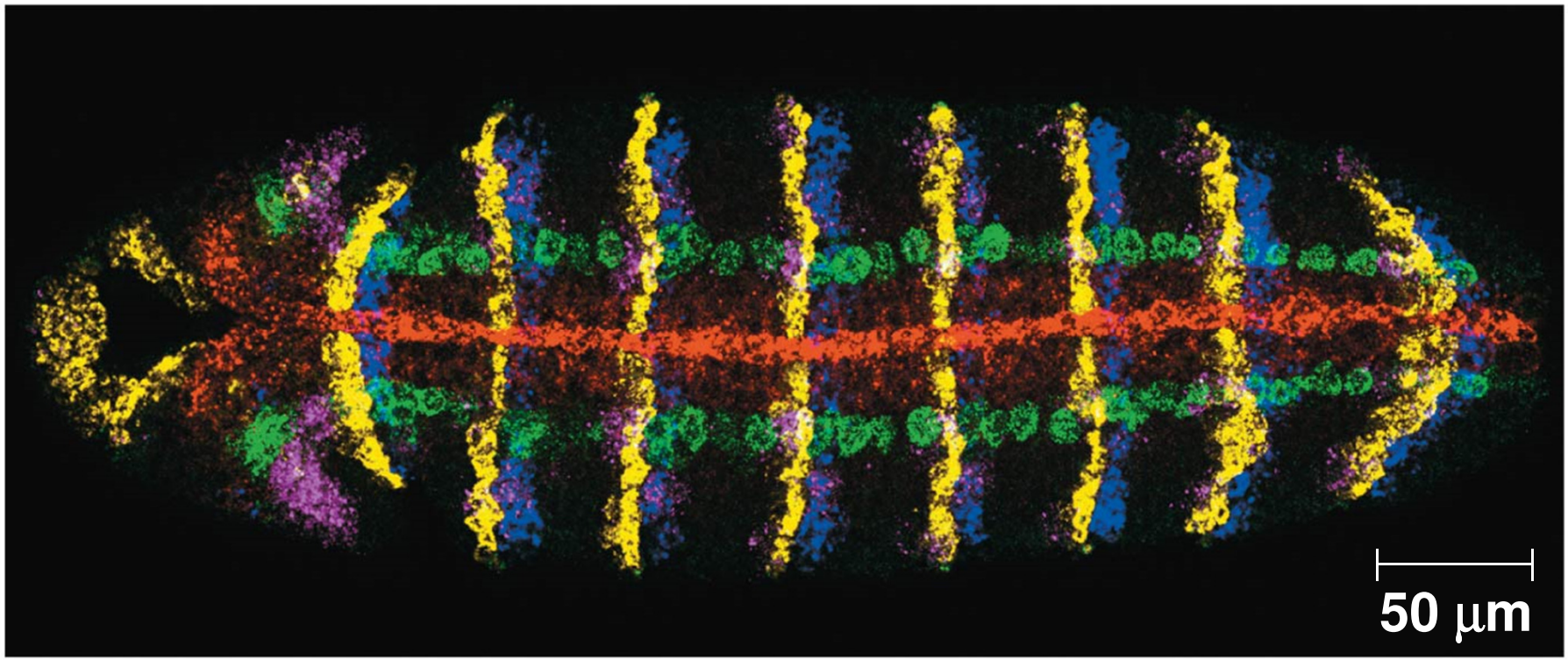
Concept 15.4: Researchers can monitor expression of specific genes

- Cells of a given multicellular organism differ from each other because they express different genes from an identical genome
- The most straightforward way to discover which genes are expressed by cells of interest is to identify the mRNAs being made

Studying the Expression of Single Genes

- We can detect mRNA in a cell using **nucleic acid hybridization**
  - Base pairing of a strand of nucleic acid to its complementary sequence
- The complementary molecule in this case is a short single-stranded DNA or RNA called a **nucleic acid probe**
  - Each probe is labeled with a fluorescent tag to allow visualization

- The technique allows us to see the mRNA in place (*in situ*) in the intact organism and is thus called ***in situ* hybridization**



- Another widely used method for comparing the amounts of specific mRNAs in several different samples is **reverse transcriptase–polymerase chain reaction** (**RT-PCR**)
  - RT-PCR turns sample sets of mRNAs into double- stranded DNAs with the corresponding sequences
  - Relies on the activity of *reverse transcriptase*
    - Can synthesize a DNA copy of an mRNA, called a **complementary DNA** (**cDNA**)
- Once the cDNA is produced, PCR is used to make many copies of the sequence of interest, using primers specific to that sequence

Figure 15.15-5

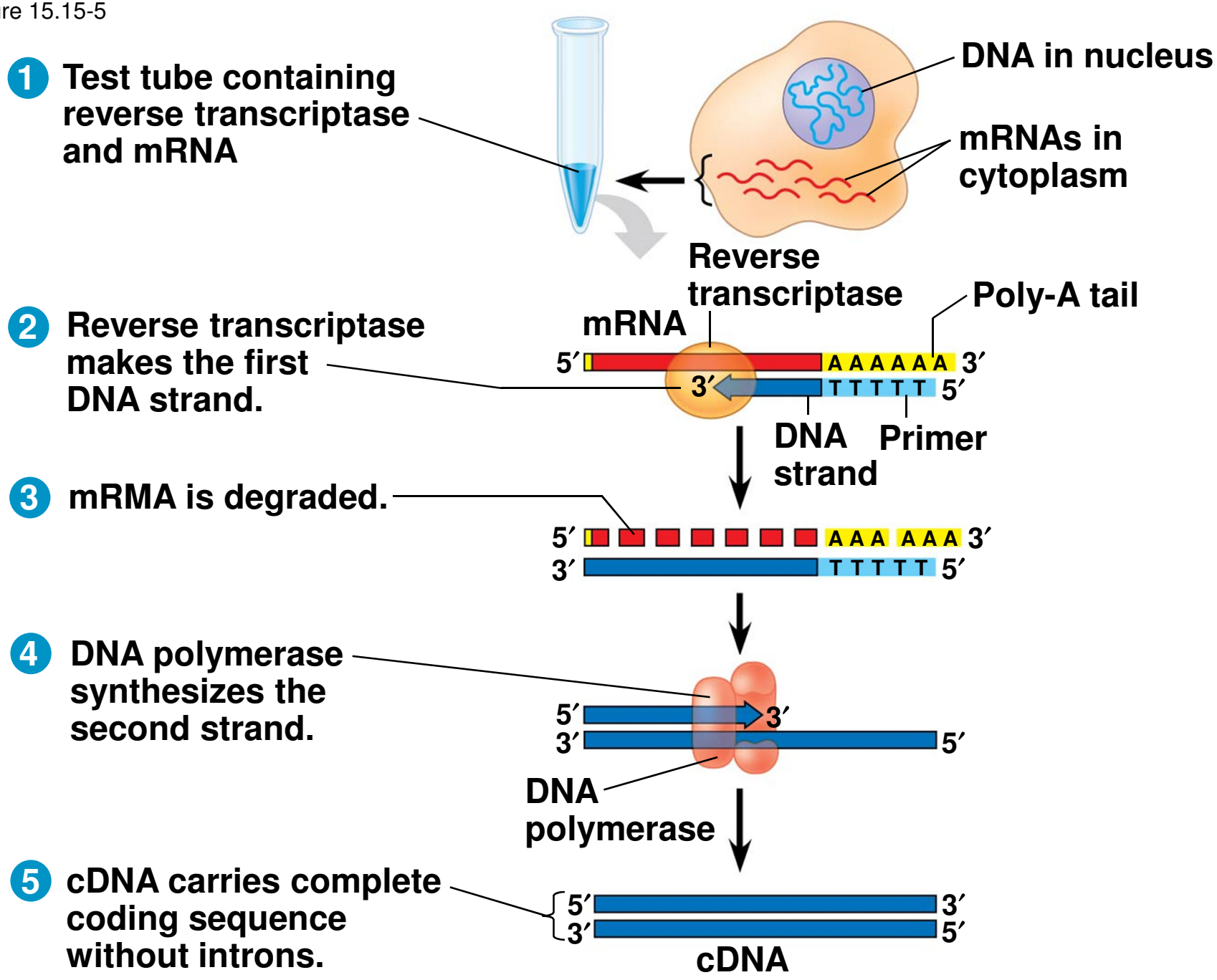
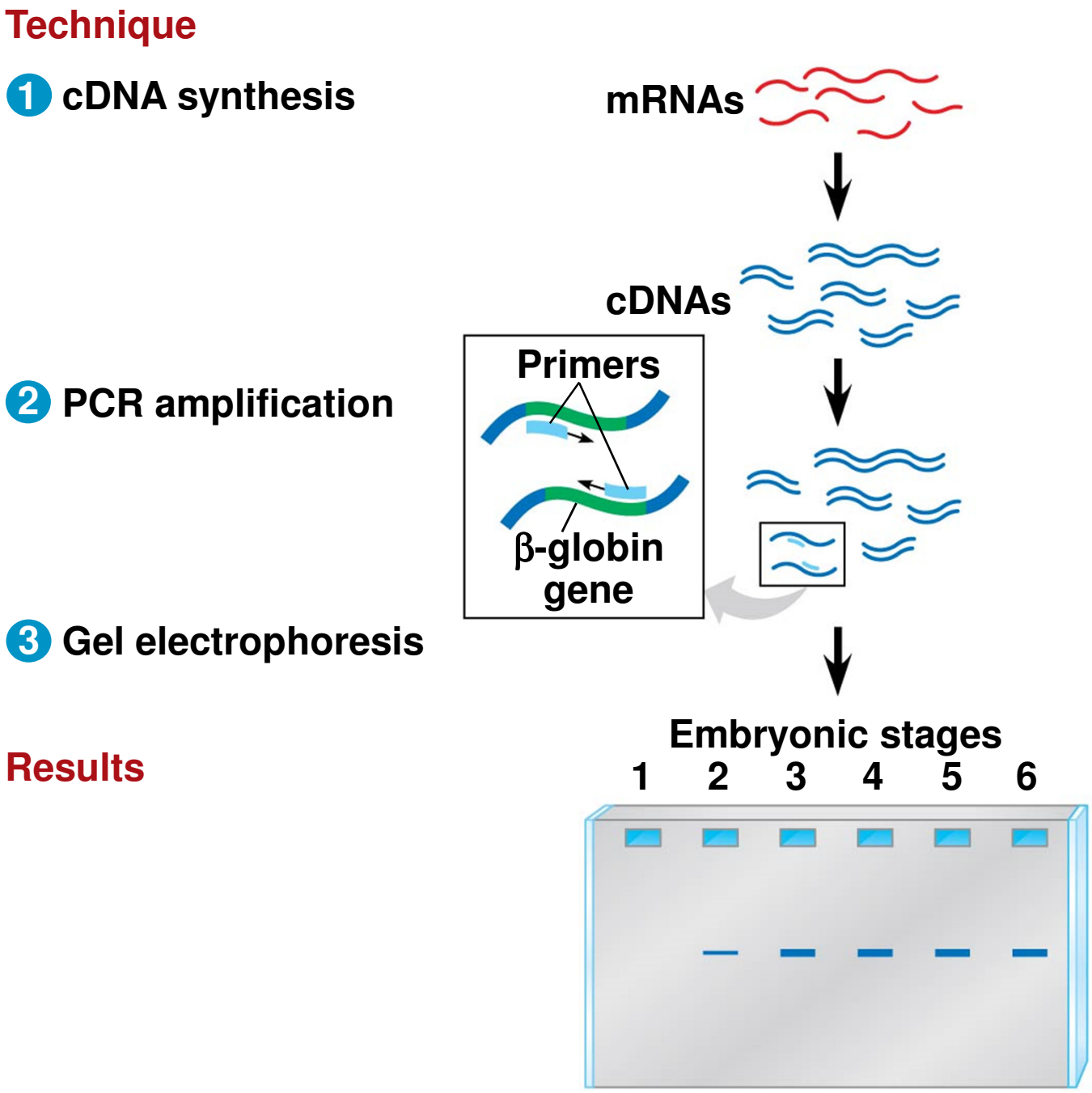


Figure 15.16

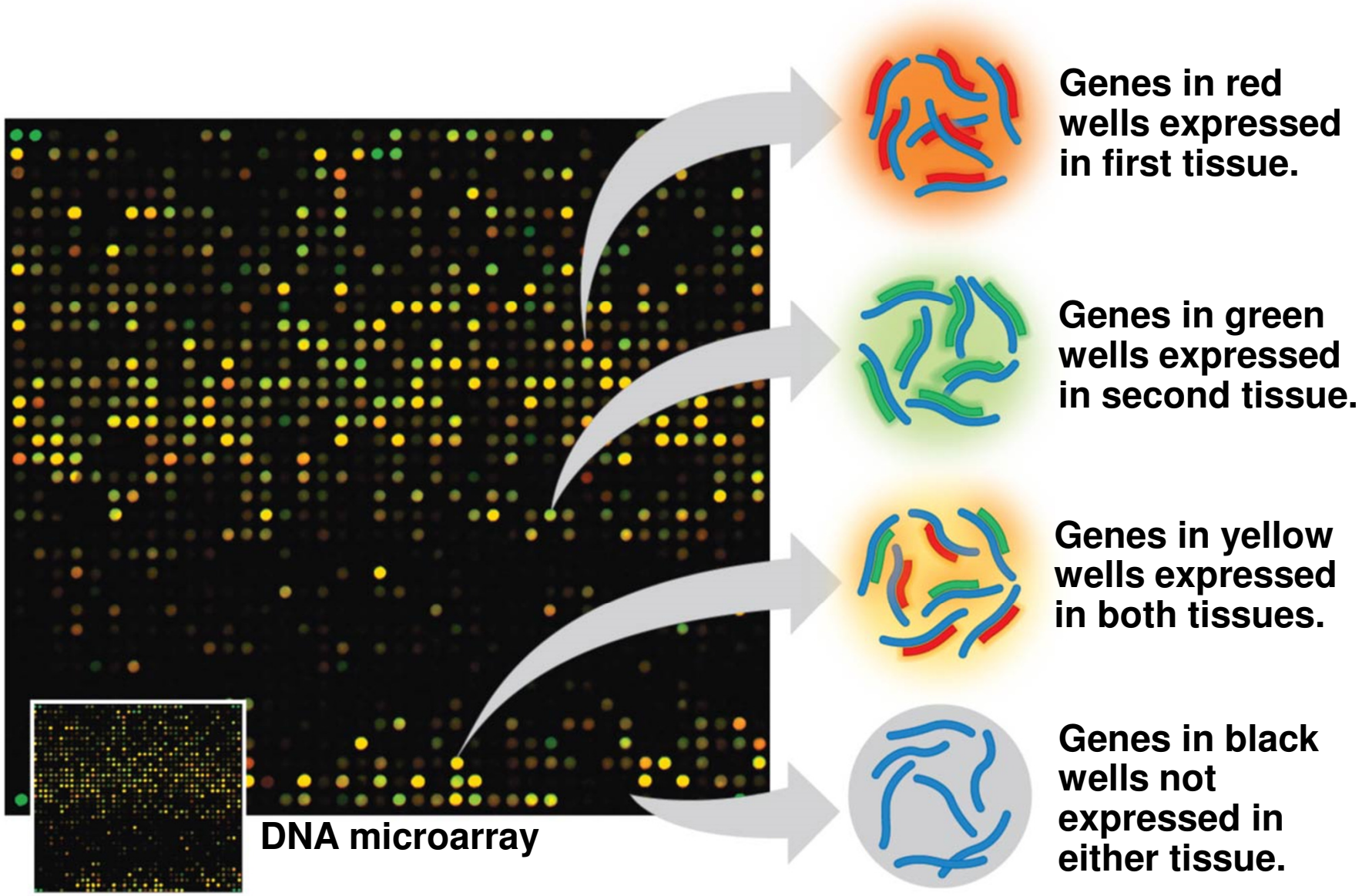

Technique
1 cDNA synthesis
mRNAs
cDNAs
2 PCR amplification
Primers
β-globin gene
3 Gel electrophoresis
Results
Embryonic stages
1 2 3 4 5 6

Studying the Expression of Groups of Genes

- A major goal of biologists is to learn how genes act together to produce and maintain a functioning organism
- Large groups of genes are studied by a systems approach
  - Allow networks of expression across a genome to be identified

- Genome-wide expression studies can be carried out using **DNA microarray assays**
  - A microarray—also called a DNA chip—contains tiny amounts of many single-stranded DNA fragments affixed to the slide in a grid
- mRNAs from cells of interest are isolated and made into cDNAs labeled with fluorescent molecules
- cDNAs from two different samples are labeled with different fluorescent tags and tested on the same microarray
- The experiment can identify subsets of genes that are being expressed differently in one sample compared to another

Figure 15.17


Genes in red wells expressed in first tissue.
Genes in green wells expressed in second tissue.
Genes in yellow wells expressed in both tissues.
Genes in black wells not expressed in either tissue.
DNA microarray

- An alternative to microarray analysis is simply to sequence cDNA samples from different tissues or stages to discover which genes are expressed
  - This is called *RNA sequencing*
  - This method is becoming more widespread as the cost of sequencing decreases
- Studies of genes that are expressed together in some tissues but not others may contribute to a better understanding of diseases and suggest new diagnostic tests or therapies